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(71)出願人　000122298
王子製紙株式会社
東京都中央区銀座４丁目７番５号

(72)発明者　刈米　昭夫
兵庫県尼崎市常光寺４丁目３番１号　新王子製紙株式会社神崎工場内

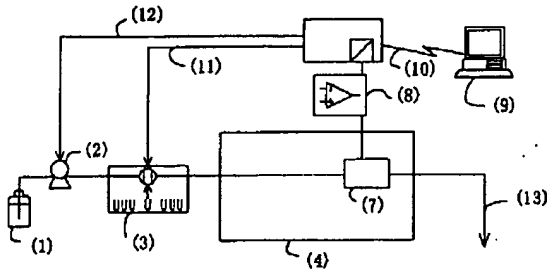
(72)発明者　原田　郁子
兵庫県尼崎市常光寺４丁目３番１号　新王子製紙株式会社神崎工場内

(72)発明者　林　隆造
兵庫県尼崎市常光寺４丁目３番１号　新王子製紙株式会社神崎工場内

(74)代理人　弁理士　三枝　英二　（外４名）

(54)【発明の名称】　固定化酵素の保存方法

(57)【要約】
【課題】固定化酵素を安定化する。
【解決手段】共有結合固定を行った酸化還元酵素の固定化酵素体を該固定化酵素体の基質酸化体と複合体を形成した状態で乾燥し、前記乾燥固定化酵素体の周囲環境中の酸素濃度を通常大気に比べて低い状態で保存することを特徴とする固定化酵素の保存方法；及び固定化酵素安定化封入体。



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【特許請求の範囲】

【請求項１】共有結合固定を行った酸化還元酵素の固定化酵素体を該固定化酵素体の基質酸化体と複合体を形成した状態で乾燥し、前記乾燥固定化酵素体の周囲環境中の酸素濃度を通常大気に比べて低い状態で保存することを特徴とする固定化酵素の保存方法。

【請求項２】基質酸化体が、アルカリ金属塩である請求項１記載の固定化酵素体の保存方法。

【請求項３】前記酸化還元酵素が酸化酵素である請求項１又は２に記載の固定化酵素体の保存方法。

【請求項４】酵素がグルコースオキシダーゼであり、基質酸化体がグルコン酸のナトリウムまたはカリウム塩であり、周囲環境中の酸素濃度を通常大気に比べて低い状態にする方法が、酸素透過率が１００ｍｌ／m^2・ａｔｍ・２４ｈｒｓ以下である素材よりなる密封可能な容器中に酵素固定化体を封入し、酸素吸収性物質を容器中に共存せしめる請求項２または３記載の固定化酵素体の保存方法。

【請求項５】酸化還元酵素の固定化酵素体と該酵素の基質酸化体との複合体を酸素濃度を通常大気に比べて低い状態にした容器中に封入してなる固定化酵素安定化封入体。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】本発明は固定化酵素を利用して、分析を行う際に固定化酵素体を安定に保存する方法および保存用の固定化酵素安定化封入体に関するものである。

【０００２】

【従来の技術】固定化酵素を利用した分析方法は臨床分野、発酵計測分野を中心に広まりつつある。特に固定化酵素を利用した分析装置として、酵素反応により増減する電極活性物質を検出するいわゆる電気化学的なバイオセンサーは操作の簡便性、精度の高さ、高度の選択性のゆえに利用数が増しつつある。特に固定化酵素体が酵素と架橋剤を反応させ、共有結合により固定化されたものである場合は、繰り返し測定の再現性に優れ、酵素の繰り返し利用による分析コストの削減が可能であるため注目されている。

【０００３】しかし固定化酵素自体は生体分子を利用したものであり、長期間使用すると活性の低下が避けられないものである。当然活性が低下した場合は固定化酵素を取り替えることになるが、従来固定化酵素の保存方法としては、適切な緩衝液中に固定化酵素を浸漬し、凍結しない程度の低温（２～１５℃）で保存することが一般的であった。この方法では固定化酵素体を保存するために場所を取る、長距離の輸送の際に水を運ぶ事になりその漏洩を防ぐために厳重な梱包が必要になる、また交換の際に作業者または作業場所がこぼれた緩衝液で汚染される、などの問題点があった。同時に緩衝液自体は微生物などの好適な培地になる可能性が高く、カビなどによる汚染により、固定化酵素自体が破壊されてしまう可能性があった。

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【０００４】上記の問題点を解決するために、一般の遊離酵素で採用されている、乾燥状態での保存が共有結合型固定化酵素で採用できれば、輸送、液の漏洩による汚染防止、微生物汚染などに対して有効な対策となり得るが、共有結合により形成された固定化酵素体を乾燥させて安定に保存する方法は従来実用化されていなかった。

【０００５】特にグルコース測定用のグルコースオキシダーゼ固定化体については、臨床分野ではベッドサイド用に用いられたり、発酵生産の生産現場で利用される例が多く、簡便な輸送、保存方法が要望されているにもかかわらず、実現していなかった。

【０００６】

【発明が解決しようとする課題】そこで本発明においては、グルコースオキシダーゼを含む酸化酵素もしくは脱水素酵素等の酸化還元酵素の酵素固定化体を安定に乾燥状態で保存する実用的方法を提供することを目的とする。

【０００７】

【課題を解決するための手段】本発明は、以下の固定化酵素体の保存方法及び固定化酵素安定化封入体を開示する。

【０００８】項１．　共有結合固定を行った酸化還元酵素の固定化酵素体を該固定化酵素体の基質酸化体と複合体を形成した状態で乾燥し、前記乾燥固定化酵素体の周囲環境中の酸素濃度を通常大気に比べて低い状態で保存することを特徴とする固定化酵素の保存方法。

【０００９】項２．　基質酸化体が、アルカリ金属塩である項１記載の固定化酵素体の保存方法。

【００１０】項３．　前記酸化還元酵素が酸化酵素である項１又は２に記載の固定化酵素体の保存方法。

【００１１】項４．　酵素がグルコースオキシダーゼであり、基質酸化体がグルコン酸のナトリウムまたはカリウム塩であり、周囲環境中の酸素濃度を通常大気に比べて低い状態にする方法が、酸素透過率が１００ｍｌ／m^2・ａｔｍ・２４ｈｒｓ以下である素材よりなる密封可能な容器中に酵素固定化体を封入し、酸素吸収性物質を容器中に共存せしめる項２または３記載の固定化酵素体の保存方法。

【００１２】項５．　酸化還元酵素の固定化酵素体と該酵素の基質酸化体との複合体を酸素濃度を通常大気に比べて低い状態にした容器中に封入してなる固定化酵素安定化封入体。

【００１３】グルコースオキシダーゼ固定化体は、血糖測定、発酵液中のグルコース測定に多用されるため、該酵素の安定化は、特に求められている。

【００１４】

【発明の実施の形態】分析用に用いられる酵素として最

も多用されるのは、電極活性物質の酸化還元を行い得る酸化酵素や脱水素酵素である。本発明はこれらの酵素を共有結合固定したものに適用される。共有結合固定は、酵素分子の本体であるタンパク質のアミノ基と反応し分子間に結合をつくる多官能基性のアルデヒド、シランカップリング剤、チオール基と反応する試薬など多くの化学試薬を利用する。たとえばグルタルアルデヒド、グリオキザールなどの多官能基性アルデヒドがその代表例である。これらの架橋を行う際に酵素タンパク質以外のタンパク質、たとえばゼラチン、血清アルブミンなどを共存させたり、酸化還元酵素以外の酵素、たとえば加水分解酵素などを共存させても良い。またタンパク質以外にも架橋後の固定化体の強度を上昇させる目的でポリリジン、ポリアリルアミンなどの合成高分子を共存させても良いし、キチン・キトサンなどの多糖類を共存させることもできる。

【００１５】酸化還元酵素としては、酸化酵素、脱水素酵素、酸素添加酵素(ﾓﾉｵｷｼｹﾞﾅｰｾﾞ,ｼﾞｵｷｼｹﾞﾅｰｾﾞ)が挙げられ、特に限定されないが、例えば、乳酸酸化酵素、乳酸脱水素酵素、グルコースオキシダーゼ、グルコース脱水素酵素、アスコルビン酸オキシダーゼ、グルタミン酸オキシダーゼ、アルコールオキシダーゼ、アルコール脱水素酵素等が挙げられる。基質酸化体は、酸化還元酵素との組合せで適宜決定される。

【００１６】好ましくは、この場合他に共存する過剰の塩類の濃度を低下させるようにするとより効果的である。たとえば乳酸酸化酵素、乳酸脱水素酵素においてはピルビン酸のナトリウム塩もしくはカリウム塩、グルコースオキシダーゼもしくはグルコース脱水素酵素においては、グルコン酸カリウムもしくはナトリウム塩を共存させると良い。この他に酵素と基質酸化体アルカリ金属塩としては、アスコルビン酸オキシダーゼに対するデヒドロアスコルビン酸ナトリウム、グルタミン酸オキシダーゼに対するα－ケトグルタル酸カリウム及びα－ケトグルタル酸ナトリウム、アルコールオキシダーゼおよびアルコール脱水素酵素に対する酢酸ナトリウム及び酢酸カリウムなどを例示できる。この基質酸化体、特にそのアルカリ金属塩類添加が保存安定性に寄与する理由は明瞭にはなっていないが、酵素の活性中心部分に基質酸化体がはまり酵素の立体構造を安定化するのではないかと思われる。基質酸化体の塩類を用いることにより基質酸化体溶液のみで緩衝作用を持たせられる。酵素の立体構造安定化の観点からは基質還元体を共存させても安定化が実現できるはずだが、一般的に酵素反応の平衡が酸化体生成側に傾いているため添加した瞬間に酸化反応が進行し好ましいものではない。特に酸化酵素では過酸化水素が生成する場合が多く、過酸化水素が酵素分子を破壊する可能性がある。

【００１７】さらに、前記乾燥固定化酵素体の周囲環境中の酸素濃度を通常大気に比べて低い状態で保存することが重要である。酸素濃度としては、通常１容量%以下、好ましくは０．５容量%以下、より好ましくは０．２容量%以下、特に０．１容量%以下が挙げられる。大気より酸素濃度を下げる方法としては袋又は容器の中に乾燥した酵素固定化体を入れ、窒素、アルゴンなどの気体を吹き込み封入したり真空にする方法などがある。より好ましい方法としては、酸素透過率が１００ｍｌ／ｍ²・ａｔｍ・２４ｈｒｓ以下である素材よりなる密封可能な容器中に酵素固定化体を封入し、酸素吸収性を有する物質を容器中に共存せしめる方法である。酸素吸収性を有する物質としては、鉄などの酸素と反応する純金属および酸素と反応する有機物などを例示できる。酸素透過率が所定の値になる容器の材質としては、ポリビニルアルコール誘導体、シリコン樹脂誘導体などの高分子を利用した袋、金属製の容器などが例示できる。

【００１８】

【実施例】以下、本発明を実施例及び比較例を用いてより詳細に説明する。

【００１９】実施例１

（１）固定化酵素体の製造方法

２ｍｍ×２ｍｍの白金作用電極と同面積の対極を形成した１０ｍｍ×１０ｍｍ、厚さ１ｍｍのアルミナセラミックス板を固定化の基体として用いた。グルコースオキシダーゼ（シグマ社、タイプII）１０ｍｇ／ｍｌ、牛血清アルブミン（シグマ社、フラクションＶ）１０ｍｇ／ｍｌを含むｐＨ７．０リン酸ナトリウム緩衝液に、最終濃度０．２%になるようにグルタルアルデヒドを加えた溶液を調製し、前記作用電極上に１０μｌ滴下し、４０℃で１５分間乾燥した。

【００２０】（２）基質酸化体アルカリ金属塩との置換

上記で作成した酵素固定化体を蒸留水中に浸漬し、固定化時に用いた緩衝液成分を洗い流し、次に１%グルコン酸カリウム水溶液に浸漬した。この処理後風乾した。

【００２１】（３）固定化酵素活性の測定

まず（１）で作成した固定化酵素体を図１のフロー型装置に装着し、グルコース溶液を注入してその感度を記録した。これを初期感度とした。

【００２２】図１のフロー型装置の概要を以下に示す。緩衝液槽（１）より緩衝液をポンプ（２）より送液し、オートサンプラー（３）よりグルコース試料溶液を１０μｌ注入した。送液された試料は、恒温槽（４）中のグルコースオキシダーゼ固定化カラム（７）を通過し、試料中のグルコースがグルコン酸に変換される。同時に生成した過酸化水素が過酸化水素電極により電流値の変化としてとらえられ、検出器（８）で検出される。

【００２３】なお、図１中、（９）はパーソナルコンピュータ、（１０）はＲＳ２３２Ｃコード、（１１）はサンプラ制御信号、（１２）は送液ポンプ制御信号を示す。

【００２４】（４）保存試験

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アルミ被覆したエバール樹脂製袋に、三菱瓦斯化学製酸素吸収剤（商品名：エージレスＺ２０）と酵素固定化体を同梱し、密封後４０℃で保存試験を行った。１０００時間保存後酵素固定化体を取り出し、（３）と同じ条件で感度を測定した。初期感度に対する保存後の感度の比をとり残留活性として表現した。

【００２５】その結果を表１に示した。１０００時間後でも８０％の活性を示し、実用上充分な耐久性を示した。

【００２６】比較例１

グルコン酸カリウムでの置換処理を行わなかった以外は実施例１と同じ方法で実験を行った。４０℃、１０００時間後の感度を表１に示す。明らかに実施例１に比べて大きな活性低下が認められる。

【００２７】比較例２

実施例１と同様にグルコン酸カリウム置換を実施し、保存時に酸素除去を行わなかった以外は全く同じ実験を行った。その結果を表１に示す。比較例１と比べてもさらに大きな活性低下が認められた。

【００２８】比較例３

グルコン酸カリウム置換を行わず、かつ保存時も酸素除去を行わずに実施例１と同じ実験を行った。その結果を表１に示す。大きな活性低下が認められた。

【００２９】比較例４

実施例１で用いたグルコースオキシダーゼをｐＨを７に調製した１％グルコン酸カリウム水溶液に１０ｍｇ／ｍｌの濃度で溶解し、そのまま４０℃で１０００時間保存した。保存前後の酵素活性を、常法に従いパーオキシダーゼを用いた過酸化水素定量法により評価した。その残留活性を表１に示した。液自体に濁りが認められ腐敗が起きたものと思われる。

【００３０】

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【表１】

	１０００時間後の残留活性％
実施例１	８０
比較例１	６５
比較例２	５０
比較例３	４０
比較例４	０

実施例１で得られた固定化グルコースオキシダーゼは、１０００時間保存後であっても十分な残留活性を有しており、安定した測定値が得られたが、比較例１～４の１０００時間保存後のグルコースオキシダーゼは、残留活性が不十分で、保存後の酵素を用いて測定したときの測定値の安定性が低下していた。

【００３１】

【発明の効果】本発明によれば、固定化酵素を長期間安定に保存することができる。

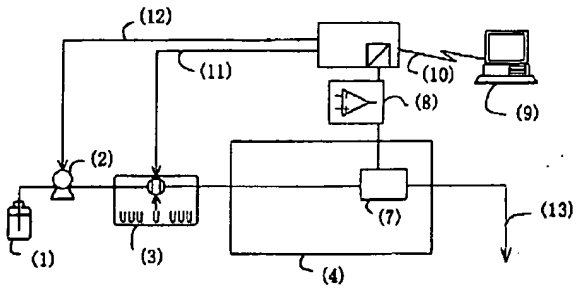
【図面の簡単な説明】

【図１】　本発明の実施例１で用いた濃度測定装置の図である。

【符号の説明】

１　緩衝液槽
２　ポンプ
３　オートサンプラー
４　恒温槽
７　酵素固定化カラム
８　検出器
９　パーソナルコンピュータ
１０　ＲＳ２３２Ｃコード
１１　サンプラ制御信号
１２　送液ポンプ制御信号

【図１】



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(71)Applicant : **OJI PAPER CO LTD**
(72)Inventor : **KARIGOME AKIO**
HARADA IKUKO
HAYASHI RYUZO

(54) PRESERVATION OF IMMOBILIZED ENZYME

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a method for preserving an immobilized enzyme, capable of preserving the immobilized enzyme stable for a long period of time by drying the immobilized enzyme material of an oxidation reduction enzyme immobilized by a coordinate bond in a state of forming a composite with the oxidized substrate material and preserving in a surrounding environment having a lower oxygen concentration than that of an ordinary atmosphere.
SOLUTION: An immobilized enzyme obtained by immobilizing an oxidation and reduction enzyme such as an oxidase or a dehydrogenase, etc., by a coordinate bond is made into a composite state with an oxidized substrate such as an alkali metal salt. After drying in the state of composite, the dried immobilized enzyme material is preserved in a surrounding atmosphere of ≤1vol% oxygen concentration, preferably ≤0.1vol.%. This method is especially useful for the preservation of an immobilized glucose oxidase frequently used for measuring a blood sugar or a glucose in a fermented liquid.

LEGAL STATUS

[Date of request for examination] 08.08.2002

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[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

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CLAIMS

[Claim(s)]
[Claim 1] The store method of the immobilized enzyme characterized by drying the immobilized-enzyme object of the oxidoreductase which performed covalent-bond fixation where the substrate oxidant and complex of this immobilized-enzyme object are formed, and usually saving the oxygen density in the circumference environment of the aforementioned dryness immobilized-enzyme object in the state of a low compared with the atmosphere.
[Claim 2] The store method of the immobilized-enzyme object according to claim 1 whose substrate oxidant is an alkali-metal salt.
[Claim 3] The store method of the immobilized-enzyme object according to claim 1 or 2 whose aforementioned oxidoreductase is oxidizing enzyme.
[Claim 4] Oxygen permeability Store method of an immobilized-enzyme object according to claim 2 or 3 which an enzyme fixed object is enclosed [store method] into the container which consists of a material which are 100 ml/m2, atm, and 24 hrses or less, and which can be sealed, and makes the oxygen-uptake nature matter live together in a container. [the method of an enzyme being a glucose oxidase, and a substrate oxidant being the sodium or potassium salt of a gluconic acid, and usually changing the oxygen density in circumference environment into a low state compared with the atmosphere]
[Claim 5] The immobilized-enzyme stabilization inclusion body which comes to enclose the complex of the immobilized-enzyme object of an oxidoreductase, and the substrate oxidant of this enzyme into the container which usually changed the oxygen density into the low state compared with the atmosphere.

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TECHNICAL FIELD

[The technical field to which invention belongs] Using an immobilized enzyme, in case this invention analyzes, it relates to the method and the immobilized-enzyme stabilization inclusion body for preservation which save an immobilized-enzyme object stably.

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PRIOR ART

[Description of the Prior Art] The analytical method using the immobilized enzyme is spreading focusing on a clinical field and a fermentation measurement field. The number of use of the electrochemical biosensor [so-called] which detects the electrode active substance fluctuated by the enzyme reaction as an analysis apparatus using especially the immobilized enzyme is increasing the height of the simple nature of operation, and precision, and on account of advanced selectivity. When especially an immobilized-enzyme object makes an enzyme and a cross linking agent react and is fixed by covalent bond, it excels in the repeatability of repeat measurement, and since curtailment of the analysis cost by repeat use of an enzyme is possible, it is observed.

[0003] However, the fall of activity will not be avoided, if the immobilized enzyme itself uses a biomolecule and it is used for a long period of time. Although an immobilized enzyme will be exchanged when activity naturally falls, as a store method of an immobilized enzyme, it was conventionally common for it to have been immersed and to have saved an immobilized enzyme at the low temperature (2-15 degrees C) of the grade which is not frozen in the suitable buffer solution. In order to carry water in the case of the long-distance transportation which takes a place since an immobilized-enzyme object is saved by this method and to prevent the disclosure, severe packing was needed, and when it was exchange, there was a trouble of being polluted with the buffer solution with which the operator or the work site fell. Simultaneously, possibility that the buffer solution itself will become suitable culture media, such as a microorganism, was high, and the immobilized enzyme itself may have been destroyed by contamination by mold etc.

[0004] Although it might become an effective cure to transportation, the pollution control by disclosure of liquid, microbial contamination, etc. when the preservation by the dryness adopted by the general free enzyme could adopt by the covalent-bond type immobilized enzyme, in order to solve the above-mentioned trouble, the method of making dry the immobilized-enzyme object formed of covalent bond, and saving stably was not put in practical use conventionally.

[0005] Especially about the glucose oxidase fixed object for glucose measurement, in a clinical field, in spite of having been used for bedsides, or there having been many examples used in the production site of production by fermentation and having demanded simple transportation and the store method, it had not realized.

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EFFECT OF THE INVENTION

[Effect of the Invention] According to this invention, an immobilized enzyme can be saved at stability for a long period of time.

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] Then, it aims at offering the practical method of saving stably the enzyme fixed object of oxidoreductases, such as oxidizing enzyme containing a glucose oxidase, or a dehydrogenase, by dryness in this invention.

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MEANS

[Means for Solving the Problem] this invention indicates the following store methods and immobilized-enzyme stabilization inclusion bodies of an immobilized-enzyme object.
[0008] Term 1. Store method of the immobilized enzyme characterized by drying the immobilized-enzyme object of the oxidoreductase which performed covalent-bond fixation where the substrate oxidant and complex of this immobilized-enzyme object are formed, and usually saving the oxygen density in the circumference environment of the aforementioned dryness immobilized-enzyme object in the state of a low compared with the atmosphere.
[0009] Term 2. Store method of the immobilized-enzyme object of the term 1 publication whose substrate oxidant is an alkali-metal salt.
[0010] Term 3. Store method of the term 1 whose aforementioned oxidoreductase is oxidizing enzyme, or an immobilized-enzyme object given in 2.
[0011] Term 4. Oxygen permeability Store method of the immobilized-enzyme object of the term 2 or 3 publications which an enzyme fixed object is enclosed into the container which consists of a material which are 100 ml/m2, atm, and 24 hrses or less, and which can be sealed, and make the oxygen-uptake nature matter live together in a container. [the method of an enzyme being a glucose oxidase, and a substrate oxidant being the sodium or potassium salt of a gluconic acid, and usually changing the oxygen density in circumference environment into a low state
[0012] Term 5. Immobilized-enzyme stabilization inclusion body which comes to enclose the complex of the immobilized-enzyme object of an oxidoreductase, and the substrate oxidant of this enzyme into the container which usually changed the oxygen density into the low state compared with the atmosphere.
[0013] Since a glucose oxidase fixed object is used abundantly at blood sugar measurement and the glucose measurement in fermented mash, especially stabilization of this enzyme is called for.
[0014]
[Embodiments of the Invention] Oxidizing enzyme and the dehydrogenase which can perform oxidation reduction of an electrode active substance are most used abundantly as an enzyme used for analysis. this invention is applied to what carried out covalent-bond fixation of these enzymes. Covalent-bond fixation uses many chemical agents, such as an aldehyde of the many functional-groups nature which reacts with the amino group of the protein which is the main part of an enzyme molecule, and builds combination between molecules, a silane coupling agent, a thiol group, and a reagent that reacts. For example, many functional-groups nature aldehydes, such as a glutaraldehyde and glyoxal, are the example of representation. In case these bridge formation is performed, protein other than enzyme protein, for example, gelatin, a serum albumin, etc. may be made to live together, or you may make enzymes other than an oxidoreductase, for example, hydrolase etc., live together. Moreover, synthetic macromolecules, such as the poly lysine and the poly allylamine, may be made to be able to live together in order to raise the intensity of the fixed object after constructing a bridge besides protein, and polysaccharide, such as chitin chitosan, can also be made to live together.
[0015] Although oxidizing enzyme, a dehydrogenase, and an oxygenase (a monooxygenase, dioxygenase)

are mentioned and it is not especially limited as an oxidoreductase, lactic-acid oxidizing enzyme, a lactate dehydrogenase, a glucose oxidase, a glucose dehydrogenase, the ascorbate oxidase, a glutamic-acid oxidase, alcohol oxidase, alcoholic dehydrogenase, etc. are mentioned, for example. A substrate oxidant is suitably determined in combination with an oxidoreductase.

[0016] It is more effective if it is made to reduce preferably the concentration of the superfluous salts which live together in this case etc. For example, it is good to make a potassium gluconate or sodium salt live together in the sodium salt or the potassium salt, glucose oxidase, or glucose dehydrogenase of a pyruvic acid in lactic-acid oxidizing enzyme and a lactate dehydrogenase. In addition, as an enzyme and a substrate oxidant alkali-metal salt, sodium acetate, potassium acetate, etc. to the dehydroascorbic-acid sodium to the ascorbate oxidase, the alpha ketoglutaric acid potassium to a glutamic-acid oxidase and alpha ketoglutaric acid sodium, alcohol oxidase, and alcoholic dehydrogenase can be illustrated. Although this substrate oxidant and especially the reason that the alkali-metal salts addition contributes to preservation stability are not clear, a substrate oxidant fits into the active-center portion of an enzyme, and it is thought that the spacial configuration of an enzyme will be stabilized. Buffer action can be given only with a substrate oxidant solution by using the salts of a substrate oxidant. Although stabilization is realized even if it makes a substrate reductant live together from a viewpoint of spacial configuration stabilization of an enzyme, oxidation reaction advances at the moment of adding, since the balance of an enzyme reaction generally leans to the oxidant generation side, and it is not desirable. By oxidizing enzyme, a hydrogen peroxide may especially generate in many cases, and a hydrogen peroxide may destroy an enzyme molecule.

[0017] Furthermore, it is important to usually save the oxygen density in the circumference environment of the aforementioned dryness immobilized-enzyme object in the state of a low compared with the atmosphere. As an oxygen density, below 0.1 capacity % is usually mentioned below 0.2 capacity % especially more preferably below 0.5 capacity % preferably below 1 capacity %. The enzyme fixed object dried in the bag or the container as a method of lowering an oxygen density from the atmosphere is put in, and there is the method of blowing and enclosing gases, such as nitrogen and an argon, or making them a vacuum etc. Oxygen permeability as a more desirable method 100 ml/m2, atm, and 24hrs It is the method of making the matter which encloses an enzyme fixed object into the container which consists of a material which is the following, and which can be sealed, and has oxygen-uptake nature living together in a container. As matter which has oxygen-uptake nature, oxygen, such as iron, the pure metal which reacts and oxygen, the organic substance which reacts can be illustrated. As the quality of the material of the container with which oxygen permeability becomes a predetermined value, the bag using macromolecules, such as a polyvinyl alcohol derivative and a silicon resin derivative, a metal container, etc. can be illustrated.

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EXAMPLE

[Example] Hereafter, this invention is explained more to a detail using an example and the example of comparison.
[0019] 10mmx10mm and the alumina-ceramics board with a thickness of 1mm in which the manufacture method 2mmx2mm platinum working electrode of an example 1(1) immobilized-enzyme object and the counter electrode of this area were formed were used as a base of fixation. To the pH 7.0 sodium phosphate buffer solution containing glucose oxidase (sigma company, Type II) 10mg/ml and a 10mg [/ml] cow serum albumin (a sigma company, Fraction V), the solution which added the glutaraldehyde was prepared and 10micro was dropped 1 times on the aforementioned working electrode so that it might become the 0.2% of the last concentration, and it dried for 15 minutes at 40 degrees C.
[0020] (2) The buffer-solution component which was immersed into distilled water and used the enzyme fixed object created by the substitution above with a substrate oxidant alkali-metal salt at the time of fixation was flushed, and, next, it was immersed in potassium-gluconate solution 1%. It was air-dry after this processing.
[0021] (3) The flow type equipment of drawing 1 was equipped with the immobilized-enzyme object created by measurement **** (1) of immobilized-enzyme activity, the glucose solution was poured in, and the sensitivity was recorded. This was made into initial sensitivity.
[0022] The outline of the flow type equipment of drawing 1 is shown below. The buffer solution was sent [tub / (1) / buffer-solution] from the pump (2), and 10microl pouring / automatic sampler / (3)] of the glucose sample solution was done. The sent sample passes the glucose oxidase fixed column (7) in a thermostat (4), and the glucose in a sample is changed into a gluconic acid. The hydrogen peroxide generated simultaneously is regarded by the hydrogen peroxide electrode as change of current value, and is detected by the detector (8).
[0023] In addition, a RS232C code and (11) show a sampler control signal, and, as for the inside of drawing 1 , and (9), a personal computer and (10) show a liquid-sending pump-control signal, as for (12).

[0024] (4) The Mitsubishi Gas Chemical oxygen absorbent (tradename : age loess Z20) and the enzyme fixed object were enclosed to the Eval resin bag manufacture which carried out retention test aluminum covering, and the retention test was performed to it at 40 degrees C after seal. The enzyme fixed object after 1000-hour preservation was taken out, and sensitivity was measured on the same conditions as (3). The ratio of the sensitivity after the preservation to initial sensitivity was taken, and it expressed as remains activity.
[0025] The result was shown in Table 1. Also after 1000 hours, 80% of activity was shown and practically sufficient endurance was shown.
[0026] It experimented by the same method as an example 1 except having not performed substitution processing by example of comparison 1 potassium gluconate. The sensitivity of after (40 degrees C and 1000 hours) is shown in Table 1. Compared with an example 1, a big activity fall is accepted clearly.
[0027] Potassium-gluconate substitution was carried out like example of comparison 2 example 1, and

the completely same experiment was conducted except having not performed deoxidation at the time of preservation. The result is shown in Table 1. Even if compared with the example 1 of comparison, the still bigger activity fall was accepted.

[0028] Example of comparison 3 potassium-gluconate substitution was not performed, and the same experiment as an example 1 was conducted, without performing deoxidation also at the time of preservation. The result is shown in Table 1. The big activity fall was accepted.

[0029] It dissolved in 1% potassium-gluconate solution which prepared pH to 7 by the concentration of 10mg/ml, and the glucose oxidase used in the example of comparison 4 example 1 was saved at 40 degrees C as it was for 1000 hours. According to the conventional method, the hydrogen-peroxide assay using the par oxidase estimated the enzyme activity before and behind preservation. The remains activity was shown in Table 1. Muddiness is accepted in liquid itself and it is thought that decomposition occurred.

[0030]

[Table 1]

	１０００時間後の残留活性%
実施例１	８０
比較例１	６５
比較例２	５０
比較例３	４０
比較例４	０

Although it has sufficient remains activity even if the fixed glucose oxidase obtained in the example 1 is after 1000-hour preservation, and the stable measured value was obtained, the glucose oxidase after 1000-hour preservation of the examples 1-4 of comparison had inadequate remains activity, and the stability of the measured value when measuring using the enzyme after preservation was falling.

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is drawing of the density measurement equipment used in the example 1 of this invention.
[Description of Notations]
1 Buffer-Solution Tub
2 Pump
3 Automatic Sampler
4 Thermostat
7 Enzyme Fixed Column
8 Detector
9 Personal Computer
10 RS232C Code
11 Sampler Control Signal
12 Liquid-Sending Pump-Control Signal

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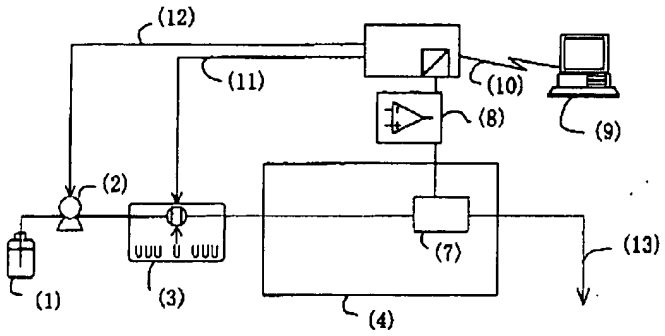
DRAWINGS

[Drawing 1]



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